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Photochemical and photocatalytic isomerization of *trans*-caffeic acid and cyclization of *cis*-caffeic acid to esculetin

F. Parrino[a,*], A. Di Paola[a], V. Loddo[a], I. Pibiri[b], M. Bellardita[a], L. Palmisano[a]

[a] *Dipartimento Energia, Ingegneria dell'Informazione e Modelli Matematici (DEIM), Università degli Studi di Palermo, Viale delle Scienze, Ed. 6, Palermo, Italy*

[b] *Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche (STEBICEF), Università degli Studi di Palermo, Viale delle Scienze, Ed. 17, Palermo, Italy*

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ABSTRACT

The photoisomerization of *trans*-caffeic acid to *cis*-caffeic acid has been studied in the presence of N_2 in homogeneous aqueous solutions and in suspensions of various TiO_2 catalysts. The results supported the hypothesis of an energy transfer process from TiO_2 to the substrate due to the recombination of the photogenerated electron–hole pairs. The differences among the measured photostationary [*cis*]/[*trans*] ratios have been attributed to the different physico-chemical properties of the catalysts. In particular, the lowest ratio measured in the presence of Merck TiO_2 was ascribed to the very low adsorption of *trans*-caffeic acid onto the surface of this sample. In the presence of O_2 and at alkaline pHs, *cis*-caffeic acid cyclized to esculetin both in the absence and in the presence of irradiation.

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1. Introduction

Absorption of light by a compound containing a double bond often results in *cis–trans* geometrical isomerization. Light energy can unpair the electrons in the π bond so that free rotation can occur about the CC bond and after rotation through 180° the unpaired electrons can pair up again forming the other geometric isomer.

The photochemical isomerization can take place through two different routes: direct or sensitized isomerization [1]. The former occurs by irradiating at a wavelength able to excite the unsaturated compound. In the case of the sensitized isomerization, the light absorbing species is a sensitizer which may induce isomerization of the unsaturated compound through electron-, energy-transfer or by means of radical chain reactions.

Direct and sensitized photochemical *cis–trans* isomerizations have been largely reported and their mechanisms are still a subject of debate [2]. Both triplet [3,4] and singlet [5,6] mechanisms have been proposed for the direct photoisomerization whereas triplet excitation of the sensitizer molecules has been suggested as the starting step for the sensitized processes [7,8].

A photostationary state is achieved when the rates of the *trans* → *cis* and *cis* → *trans* reactions are equal. The isomeric composition depends on the relative spectral absorption of the two isomers and on the relative quantum yield of their photochemical conversion [9]. The photostationary [*cis*]/[*trans*] ratio can be calculated from the following relationship [8,9]:

$$\frac{[cis]}{[trans]} = \frac{\Phi_t}{\Phi_c}\frac{\epsilon_t}{\epsilon_c} \quad (1)$$

where Φ_t and Φ_c are the quantum yields for the *trans* → *cis* and *cis* → *trans* reactions and ϵ_t and ϵ_c are the molar extinction coefficients of the two isomers. Use of an appropriate sensitizer allows to enhance the *cis* or *trans* isomer proportion at the photostationary state [3].

Although the photo-isomerization process is a well studied phenomenon, it appears to be an ever-green topic considering the outstanding importance of optical storage data systems in the today's knowledge-based society. In fact, the two forms assumed by the same molecule under light exposure can function as molecular switch in nanotechnological applications.

The photochemical isomerization of cinnamic acids has received considerable attention [10–17]. These acids can exist in the *cis* or *trans* form owing to the presence of a vinyl group in the side chain. Williams [12] found the presence of both *cis* and *trans* isomers by paper chromatography of cinnamic acid derivatives obtained by irradiation with UV light. Kahnt [15] reported that irradiated

* Corresponding author.
E-mail address: francesco.parrino@unipa.it (F. Parrino).

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aqueous solutions of hydroxycinnamic acids reached a constant *trans–cis* photostationary state and conversion was maximized between pH 5.0–7.0. Gas chromatography [14] and high performance liquid chromatography [18] were used for the separation and estimation of the *cis* and *trans* isomers of the acids.

Caffeic acid is an *ortho*-diphenol belonging to the family of the cinnamic acids. It is one of the most widely distributed compounds in the plant tissues as it takes part to the biosynthesis of lignin and flavonoids [19]. Caffeic acid has attracted attention for its wide variety of biological properties as anti-oxidant, anti-viral, anti-inflammatory, anti-rheumatic, as inhibitor of carcinogenesis [20] and as bactericide at alkaline pH [21]. Based on these properties a lot of studies has been done to develop caffeic acid-based drugs. Being caffeic acid virtually omnipresent in the plant kingdom, it is therefore present in all plant-derived food systems. The auto [22] or enzymatic [23,24] natural oxidation to *o*-quinone derivatives of caffeic acid-like compounds produces chain reactions responsible for the browning of natural products especially at alkaline pHs [20].

Several studies have reported the *trans–cis* photoisomerization of caffeic acid [15–18,25] and its photochemical conversion to esculetin [25,27–29]. Recent papers have concerned the photodegradation of caffeic acid in aqueous solution [25,26] and in the presence of TiO_2 [17,25,30–32].

The aim of this work was to investigate the effects of UV light and various TiO_2 catalysts on the photoisomerization of *trans*-caffeic acid (at different pHs). The reaction was carried out in virtual absence of oxygen so that other photocatalytic side reactions were depressed and it was possible to distinguish the contribution of electron transfer, energy transfer or radical chain reactions. Furthermore, the cyclization of the *cis* isomer to esculetin, which occurs in the presence of oxygen, is discussed throughout the paper and the influence of the reacting conditions was analyzed.

2. Experimental

Trans-caffeic acid was obtained from Sigma–Aldrich (98%) and used as received without any further purification. The photoreactivity experiments were carried out by using two commercial TiO_2 samples, i.e. Degussa P25 (ca. 80% anatase and 20% rutile, BET specific surface area (SSA): 50 $m^2\,g^{-1}$) and Merck (100% anatase, SSA: 10 $m^2\,g^{-1}$), and a home-prepared TiO_2 sample, denoted as HP0.5 (SSA: 235 $m^2\,g^{-1}$). HP0.5 was prepared by slowly adding 5 mL of $TiCl_4$ to 50 mL of water in a beaker, under magnetic stirring. After that, the beaker was closed and stirring was prolonged for 12 h at room temperature, obtaining a clear solution. Afterwards, the solution was boiled at 100 °C for 0.5 h. This treatment produced a milky white suspension that was then dried at 150 °C in order to obtain the final solid, consisting mainly of amorphous TiO_2 and crystals of anatase (75%) and rutile (25%). Al_2O_3 (Fluka type 507C neutral, for chromatography) was used for some blank experiments. A silylated P25 TiO_2 sample (labeled as P25-Si) was obtained as follows: in a round bottom flask 1 g of P25 was mixed with 50 mL of *n*-hexane, 2 mL of triethylamine and 7 g of $C_{16}H_{32}Si(OCH_3)_3$ (Wacker Silan 250, 2VP). The suspension was boiled at 90° C for 24 h. The solid phase was separated by centrifugation, washed three times with *n*-hexane, and finally dried at room temperature under vacuum.

The irradiation experiments were carried out in a cylindrical photoreactor (internal diameter: 32 mm, height: 188 mm) containing 150 mL of aqueous suspension, irradiated by six external Actinic BL TL MINI 15 W/10 Philips fluorescent lamps emitting in the 340–420 wavelength range with the main emission peak at 365 nm. The reactor was cooled by water circulating through a Pyrex thimble, so that the temperature of the suspension was about 30 °C. The radiation energy impinging on the suspension was measured by a radiometer Delta Ohm DO9721 with an UVA probe: its average value was 0.65 mW cm^{-2}. Nitrogen or oxygen were continuously bubbled for 0.5 h before switching on the lamps and throughout the runs. The initial *trans*-caffeic concentration was 0.35 mM and the pH was adjusted to 3.5, 6.0 and 8.0 using a dilute NaOH solution. The amounts of catalyst used for the runs were 0.5, 0.6 and 0.8 g L^{-1} for P25, HP0.5 and Merck, respectively. These amounts were chosen in order to ensure that almost all the photons emitted by the lamps were absorbed by the suspension.

Samples of the irradiated solution were withdrawn at fixed times and immediately filtered through 0.25 μm membranes (HA, Millipore) to separate the catalyst particles. The quantitative determination of *trans*-caffeic acid, *cis*-caffeic acid and esculetin was performed by means of a HPLC Beckman Coulter (System Gold 126 Solvent Module and 168 Diode Array Detector), equipped with a Phenomenex Kinetex 5 mm C18 100A column (4.6 mm × 150 mm) working at room temperature. The eluent consisted of a mixture of acetonitrile and 1 mM trifluoroacetic acid aqueous solution (15:85 volumetric ratio) and the flow rate was 0.6 mL min^{-1}. Absorbance was measured at 260 nm. Species were identified by comparing their retention times and UV–vis spectra with those of standards.

The calibration curve of *cis*-caffeic acid, which is not commercially available, was obtained as follows: a solution of *trans*-caffeic acid was irradiated under UV light and under nitrogen atmosphere for 20 min in order to transform it partially into *cis*-caffeic acid. Every 5 min, samples were withdrawn and immediately analyzed. HPLC analysis revealed that the samples consisted of only *cis*- and *trans*-caffeic acid without appreciable traces of oxidation by-products. TOC analysis revealed a constant organic carbon content of the samples indicating that no CO_2 was produced during this short irradiation time. The concentration of the photoproduced *cis*-caffeic acid was calculated as the difference between the initial and the residual concentration of *trans*-caffeic acid. The obtained calibration curve was then used for all long lasting runs. The reaction was scaled up to mg quantities in order to isolate and characterize the reaction products.

Preparative thin layer chromatography using Merck PLC silica gel 60 F254 2 mm glass plate 20 × 20 cm and ethyl acetate as eluent permitted to partially separate *trans*-caffeic acid from the mixture of *cis* and *trans*-caffeic acid. This mixture enriched in *cis*-caffeic acid was identified by NMR analysis. ^{1}H NMR spectra (250 MHz) and ^{13}C NMR spectra (62.7 MHz) were acquired by a Bruker AC 250E spectrometer. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 7.31 (1H, brs, H-2′), 6.98 (1H, br d J = 8.1 Hz, H-6′), 6.66 (1H, d J = 8.1 Hz, H-5′), 6.45 (1H, d J = 12.8 Hz, H-2), 5.68 (1H, d J = 12.8 Hz, H-3); ^{13}C NMR (DMSO-d_6, 62.7 MHz) δ 168.9 (COOH), 146.4 (C-4), 144.6 (C-β), 137.5 (C-3), 126.8 (C-1), 122.5 (C-6), 119.7 (C-2), 117.4 (C-α), 114.9 (C-5).

The molecular structures of the various species present in solution were obtained by using the Density Functional Theory. The computations were performed with the B3LYP hybrid function and 6-31G** basis set using the Gaussian 03 program package [33].

3. Results and discussion

3.1. Trans-*caffeic acid photoisomerization*

Fig. 1 shows the reaction of photoisomerization of *trans*-caffeic acid to *cis*-caffeic acid.

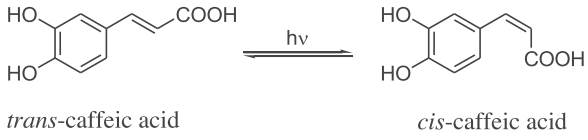

Fig. 1. Photoisomerization of *trans*-caffeic acid.

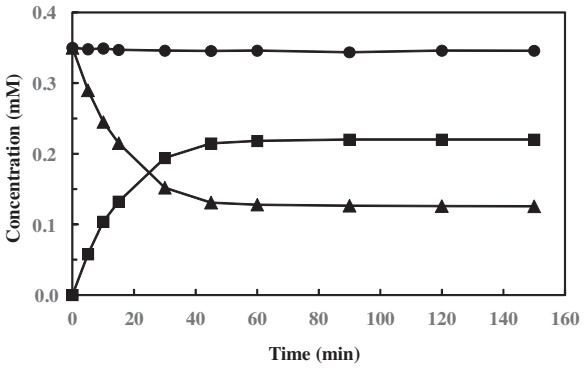

Fig. 2. Time-course of the concentrations of *trans*-caffeic acid (▲), *cis*-caffeic acid (■) and total caffeic acid (●) under UV irradiation, at pH 8.0. Initial *trans*-caffeic acid concentration: 0.35 mM. Estimated efficiency of the radiation arrived to the reactor after 30 min: 0.08 mol of photoisomerizated molecules/Einstein of photons.

Table 1
Photostationary [*cis*]/[*trans*] ratios reached starting from a 0.35 mM *trans*-caffeic acid solution, under nitrogen atmosphere. The initial pH values were fixed at 3.5, 6.0 and 8.0.

	Homogeneous solution	P25	HP0.5	Merck	Al_2O_3	P25-Si
pH 3.5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
pH 6.0	2.0	1.8	2.3	1.5	0.4	2.2
pH 8.0	1.8	2.0	2.3	1.5	0.7	2.1

All the experiments described in this section were performed under continuous bubbling of nitrogen through the suspension so that the oxygen concentration in the solution could be neglected.

The photoisomerization of *trans*-caffeic acid under UV irradiation was followed at pH 3.5, 6.0 and 8.0. The pH was adjusted after addition of the catalysts. Preliminary tests were carried out in order to verify the stability of the substrate since auto-oxidation of caffeic acid occurs at high pH values [22]. *Trans*-caffeic acid solutions were left in the dark at pH 8 and samples were taken at fixed times. The concentration did not change practically during the first 6 h.

Fig. 2 shows a representative run carried out in the absence of catalyst at pH 8. The isomerization of *trans*-caffeic acid occurred immediately after switching on the lamp as evidenced by the immediate formation of the *cis* isomer. In the afore mentioned conditions, photostationary concentrations of *trans*- and *cis*-caffeic acid were reached after ca. 60 min. The total concentration of caffeic acid remained almost constant during the run and only traces of esculetin were detected by HPLC analysis. The trend of the concentrations of *cis*- and *trans*-caffeic acid during irradiation was the same in the absence or in the presence of all the catalysts considered in this work. Notably, the photostationary state values always showed a higher amount of *cis*-caffeic acid.

Table 1 reports the values of the [*cis*]/[*trans*] ratios measured after photostationary states were established. The pH increased during the photoisomerization and, in particular, more than one unity increase was measured in the solution with initial pH 6.0.

At pH 3.5 very low conversions to *cis*-caffeic were found, in agreement with the results of Kahnt [15]. At pH 8.0, the [*cis*]/[*trans*] ratios measured in the presence of P25 and HP0.5 were higher than that obtained by irradiation of the homogeneous *trans*-caffeic acid solution. This indicates that these catalysts participate to the isomerization process which occurs through two synergistic parallel pathways: (i) photoisomerization in homogeneous phase and (ii) TiO_2-mediated photocatalytic isomerization [34–37].

Blank tests in the dark in the presence of P25 or HP0.5 did not give rise to any isomerization so that a simple acid/base catalyzed isomerization can be excluded and the presence of UV light appears indispensable. However, this does not necessarily imply that the band gap excitation of the two catalysts is responsible for the enhanced isomerization.

Sclafani et al. [38] attributed the photo-induced decarboxylation of ethanoic acid on silica to the light action on the weakened ethanoate-like species adsorbed on the silica surface without excitation of silica. A similar mechanism could occur for the *trans*-caffeic acid isomerization as this molecule is reported to have strong interaction with P25 TiO_2 due to the presence of the two phenolic hydroxyl groups and the CC double bond of the acrylic group [39]. To prove this hypothesis, isomerization runs were carried out in the presence of Al_2O_3. In this case, the measured [*cis*]/[*trans*] ratio was significantly lower than that obtained in homogeneous phase. This means that the contemporaneous action of adsorption and light was not enough to promote isomerization. In practice, Al_2O_3 only exerts a screening effect of light, being its excitation impossible due to its wide band gap. It is worth to remember that the suspensions of the various samples absorbed all the photons emitted by the lamps so that, in the absence of a catalytic role, the behavior of P25, HP0.5 and Al_2O_3 would have been the same. Instead, the TiO_2 samples behaved as heterogeneous photosensitizers. Although it is difficult to estimate the contribution of the TiO_2-induced process to the isomerization process, an indication may be obtained from the difference between the photostationary [*cis*]/[*trans*] ratios reached in the presence of Al_2O_3 and TiO_2.

The first step of the photocatalytic isomerization of *trans*-caffeic acid is the light excitation of TiO_2. The photogenerated electrons and holes migrate to the surface of the catalyst where they (i) may react with water molecules, producing radicals, (ii) may undergo interfacial electron transfer with adsorbed substrates, or (iii) may recombine releasing non-radiative energy.

The first possibility implies that the caffeic acid isomerization proceeds via a radical chain induced mechanism. Control experiments allowed to exclude this pathway since the isomerization did not continue if light was turned off after 1 h of irradiation and the suspension was left under continuous stirring for other 3 h in the dark.

As afore mentioned, the photocatalytic isomerization may proceed through electron transfer or through energy transfer. Distinguishing between these two mechanisms is difficult since both processes may occur at the same time affording the same products.

Oh et al. [37] proposed that the *cis–trans* photoisomerization of maleic and fumaric acid occurred by way of a reductive electron transfer from TiO_2 to the adsorbed acids, followed by a bond rotation of the three electrons CC bond and the final return of the electron to TiO_2 or another species which reset the stereochemistry of the double bond. This mechanism is unlikely for the photocatalytic isomerization of caffeic acid because it is in contradiction with the results obtained by adding 2-propanol (2% v/v) to the suspension of P25.

As shown in Fig. 3, the [*cis*]/[*trans*] ratio measured in the presence of 2-propanol was very low for 60 min and successively it slowly increased toward the stationary value very similar to that obtained in the absence of 2-propanol. The presence of a hole scavenger should enhance the availability of electrons. The low isomerization efficiency observed in the presence of 2-propanol allows to exclude an electron transfer dependent isomerization. On the other hand, the photoisomerization of *trans*-caffeic acid occurred in the absence of oxygen so that the photogenerated electrons were only scarcely scavenged by TiO_2.

Recent studies have shown that modifications of the TiO_2 surface with fluoride ions or silyl groups enhanced the energy transfer pathway at the expense of the interfacial electron transfer [40]. Control experiments were carried out with a surface modified catalyst to verify if the energy transfer was responsible for the photocatalytic isomerization of *trans*-caffeic acid. Modification of TiO_2 by substitution of the surface —OH groups with F^- ions was not

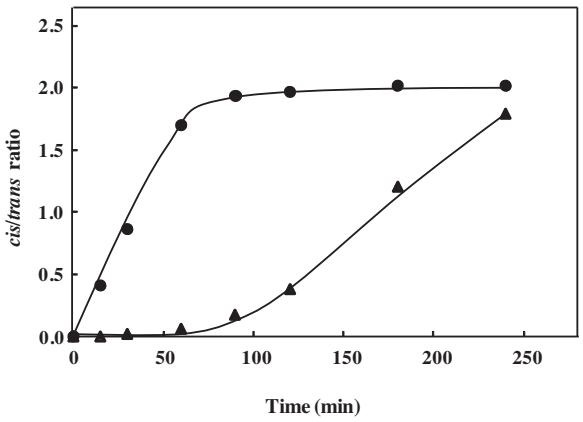

Fig. 3. Time course of the [*cis*]/[*trans*] ratio measured in the presence of P25 under UV irradiation with (▲) and without 2-propanol (●). Initial *trans*-caffeic acid concentration: 0.35 mM; pH 8.0; 2-propanol: 2% v/v.

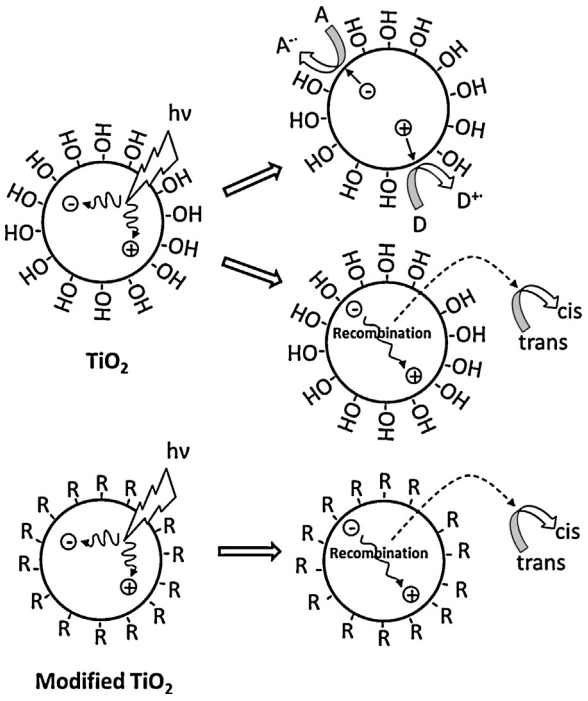

Fig. 4. Alternative photocatalysis processes at TiO_2 (upper scheme) or at surface modified TiO_2 (lower scheme). D and A represent donor and acceptor species, respectively.
(Adapted from Jańczyk et al. [40].)

practicable because the solution became strongly colored. Silylation of P25 gave a highly hydrophobic catalyst with surface—OH groups exchanged by silyl groups terminating with long alkyl chains. As reported in Table 1, the [*cis*]/[*trans*] ratio measured in the presence of P25-Si was slightly higher than that obtained with bare P25. Differently from Al_2O_3 that is an insulator, P25-Si favored the isomerization of caffeic acid.

Fig. 4 shows the mechanisms of the photocatalytic processes that can occur in the presence of P25 and HP0.5 or P25-Si. The described results support the hypothesis of an energy transfer mechanism for the photocatalytic isomerization of *trans*-caffeic acid. All the experiments were carried out in the absence of oxygen and only *cis*- and *trans*-caffeic acid were detected at the end of each run. The lack of other reaction products indicates that isomerization was the only process induced by the light, so that no reduction or oxidation of species present in solution occurred by

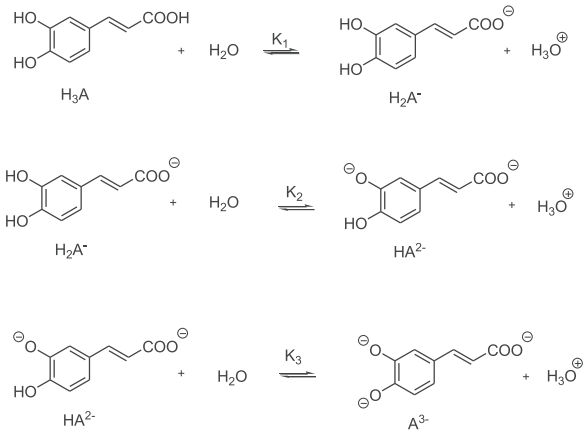

Fig. 5. Dissociation equilibria of caffeic acid.

interfacial transfer of electrons and/or holes. This means that the recombination was the most probable fate of the photogenerated electron/hole pairs. Silylation of the TiO_2 surface suppresses the possibility of the electron transfer process but allows the transmission of energy caused by the electron–hole pair recombination.

The isomerization ratio measured in the presence of HP0.5 was higher than the value obtained with P25. Although P25 and HP0.5 contain similar amounts of anatase and rutile, the former sample is much more crystalline and has a much lower specific surface area. Ohtani et al. [41] attributed the negligible photocatalytic activity of amorphous TiO_2 samples to the recombination of photoexcited electrons and holes at defects located on the surface and in the bulk of the particles. The higher is the percentage of amorphous phase, the greater is the amount of imperfections as impurities, dangling bonds, or microvoids, that behave as recombination centers for the electron/hole pairs. As reported in a previous paper [42], the percentages of crystallinity are 90% for P25 and only 9% for HP0.5. The irradiation results confirm that a facilitated recombination of electrons and holes results in a greater isomerization efficiency of *trans*-caffeic acid.

Table 1 shows that the photostationary [*cis*]/[*trans*] ratio reached in the presence of Merck TiO_2 was lower than the corresponding values established both in homogeneous phase and with the other TiO_2 samples. FTIR investigations found that ferulic acid (a hydroxycinnamic acid which differs from caffeic acid for a methoxy group instead of a hydroxyl in position 3) was not adsorbed on the surface of Merck TiO_2 [43]. Similarly, dark adsorption tests revealed a very low adsorption of *trans*-caffeic acid onto the surface of the Merck sample. Fast field cycling NMR experiments evidenced that water molecules chemically interact with the Merck surface through H—bond formation with the anatase sample [44]. This means that the molecules of *trans*-caffeic acid are not able to reach the surface of Merck TiO_2 which is covered by a shell of water molecules.

The significant difference between the [*cis*]/[*trans*] ratios reached in the presence of Merck TiO_2 and Al_2O_3 supports the hypothesis that an energy transfer process occurs in the suspensions of Merck TiO_2. Anyway, a part of the energy obtained through charge recombination is lost through vibrations of the water molecules adsorbed on the surface and consequently, the synergistic action of Merck TiO_2 is reduced with respect to that of the other TiO_2 samples.

3.2. Influence of pH on the photoisomerization

Variations of pH control the ionization states of caffeic acid and of the TiO_2 surface, so that changes in their interactions are

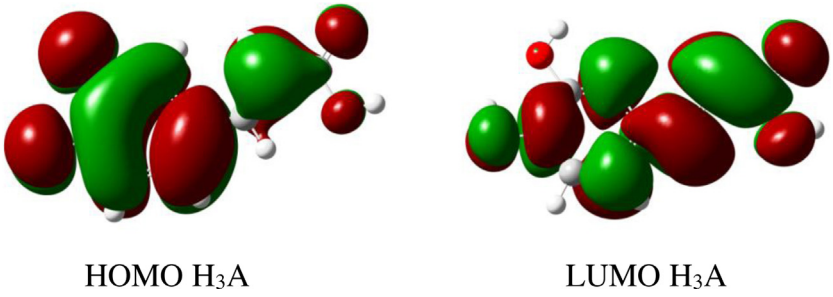

Fig. 6. HOMO and LUMO molecular orbital surfaces of caffeic acid.

Table 2
Percentages of anions and undissociated *trans*-caffeic acid present in solution at various pHs.

pH	H_3A	H_2A^-	HA^{2-}	A^{3-}
3.5	90%	10%	–	–
6.0	3%	97%	–	–
8.0	–	83%	17%	–

Table 3
Calculated energies gaps between HOMO and LUMO orbitals of the species present in aqueous solution.

pH	ΔE_{calcd} (eV)
3.5	4.14 (H_3A)
6.0	2.67 (H_2A^-)
8.0	3.89 (HA^{2-})

possible. The increased conversion of *trans*-caffeic acid at pH 6.0 in the absence of catalysts, is in agreement with the results of Kahnt [15] who found the highest percentage of *cis*-caffeic acid at pH 5.0–7.0 while the least conversion occurred at pH 3.5 and 7.8.

Caffeic acid is a triprotic acid with three dissociation equilibria steps shown in Fig. 5.

The dissociation of caffeic acid in water gives rise to three different anions. The values of pKa reported by Lamy et al. [45] are 4.45, 8.66 and 11.8, respectively. Table 2 reports the percentages of the various species present in solution at pH 3.5, 6.0 and 8.0.

HOMO and LUMO molecular orbitals energies of the various species present in solution have been determined by the Density Functional Theory. The energies gaps calculated at various pH values, are reported in Table 3.

The data show that the H_3A species presents the highest HOMO–LUMO energy gap, while the value associated with the H_2A^- species is the lowest. These results allow to hypothesize that at acidic pH the photoisomerization is very low because of the high energy gap associated to the HOMO–LUMO transition of the protonated species. At pH 6.0 the prevalent H_2A^- species absorbs the exciting radiation and the excited state gives rise to the photoisomerization product in high yield. At pH 8.0, the decrement of H_2A^- concentration corresponds to the formation of HA^{2-} species, that presents a higher HOMO–LUMO energy gap, thus confirming that the photoisomerization is mainly due to the H_2A^- species.

Fig. 6 shows the HOMO and LUMO molecular orbitals of caffeic acid. The corresponding orbitals of the H_2A^- anion and HA^{2-} dianion have not been reported because their shapes are identical. For all the protonated and deprotonated species the LUMO orbital presents a node in the double bond that is not present in the corresponding HOMO orbital. This confirms that the isomerization of the double bond can occur only in the excited state.

As shown in Table 1, the [*cis*]/[*trans*] ratios obtained at pH 6.0 and 8.0 in the presence of HP0.5, Merck and P25-Si were practically the same whereas the value obtained at pH 6.0 in the presence of P25

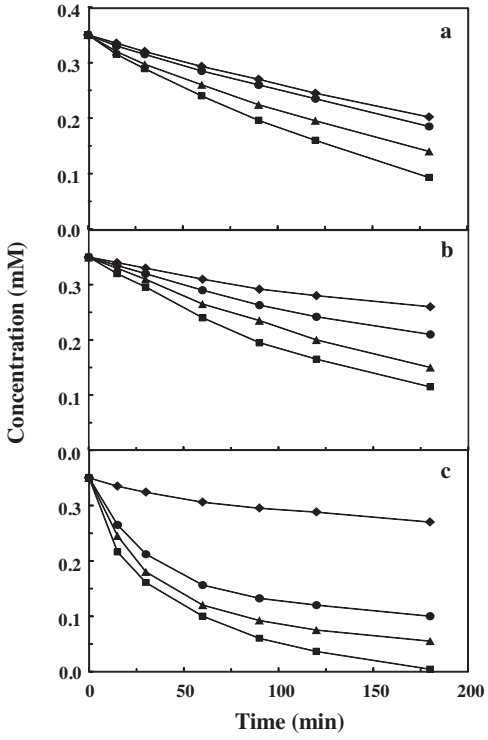

Fig. 7. Concentration profiles of total caffeic acid determined under UV irradiation with O_2 in the absence (♦) and in the presence of: Merck TiO_2 (●), P25 (▲) and HP0.5 (■). (a) pH 3.5; (b) pH 6.0; (c) pH 8.0.

was lower than that measured at pH 8.0. This last result can be justified by taking into account the point of zero charge (pzc) of P25 and the influence of pH on the adsorption of caffeic acid. Table 2 shows that caffeic acid is almost completely dissociated at pH 6.0 and 97% of the H_2A^- anion is present in solution. The pzc values of P25 found in literature generally range from 6.2 to 6.9 (although lower values as 5.2 [46] and 4.0 [47] have been also reported). At pH 6.0 < pzc, the surface of TiO_2 is slightly positively charged providing a favorable condition for the H_2A^- ions to approach. At pH 8.0 > pzc, the surface of TiO_2 is negatively charged and the adsorption of H_2A^- (83%) and of HA^{2-} (17%) ions becomes very difficult. Raman and IR spectroscopies evidenced that the adsorption of caffeic acid on P25 occurs through the interaction with the two phenolic hydroxyl groups and with the double CC bond of the acrylic group [39]. Obviously, the electrostatic attraction between double bond and surface of the catalyst prevents the free rotation around the CC bond and consequently only very slightly adsorbed and/or not adsorbed species which are located close to the surface can isomerize. This hypothesis seems confirmed in the case of HP0.5, whose pzc value reported in literature [47] is 0.3. In fact, at pH 6.0 and pH 8.0 the surface

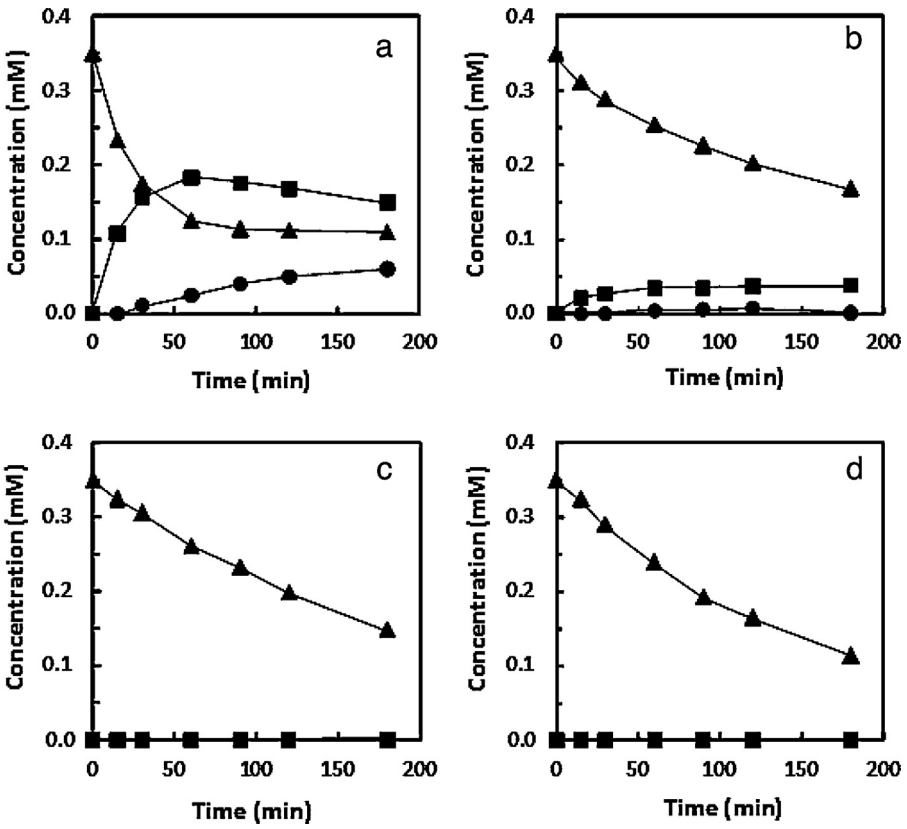

Fig. 8. Concentration profiles of *trans*-caffeic acid (▲), *cis*-caffeic acid (■) and esculetin (●) determined under UV irradiation with O_2 at pH 6.0 in the absence (a) and in the presence of Merck TiO_2 (b), P25 (c) and HP0.5 (d).

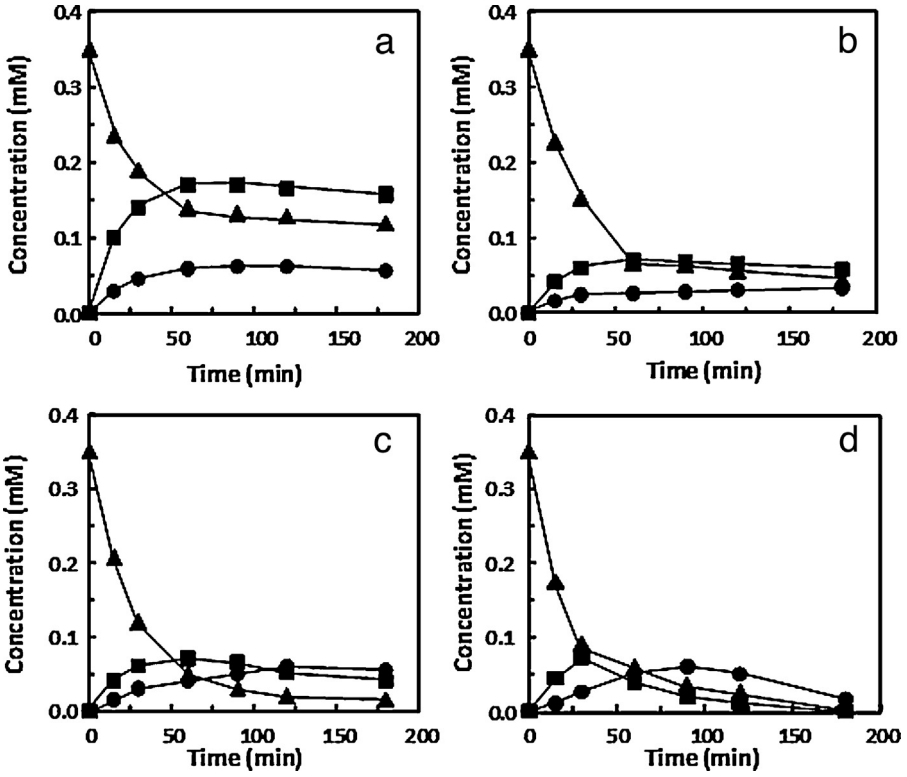

Fig. 9. Concentration profiles of *trans*-caffeic acid (▲), *cis*-caffeic acid (■) and esculetin (●) determined under UV irradiation with O_2 at pH 8.0 in the absence (a) and in the presence of Merck TiO_2 (b), P25 (c) and HP0.5 (d).

of HP0.5 is strongly negatively charged and the [*cis*]/[*trans*] ratios measured at both pHs were equal.

3.3. Trans-*caffeic acid photodegradation and cyclization to esculetin*

The experiments described in this paragraph were performed continuously bubbling oxygen throughout the irradiation. Differently from the runs carried out in the presence of N_2, the total caffeic acid concentration decreased with time due to the formation of esculetin (6,7-dihydroxycoumarin) and/or other oxidations products which were not recognized. In particular, Grimes et al. [17] identified 3,4-dihydroxybenzaldehyde and 3,4-dihydroxybenzoic acid as degradation products, whereas Le Person et al. [25] also reported the likely formation of vinylcathecol. As already found by Cilliers and Singleton [20,22], especially at non acidic pH values, browning of the solutions occurred in the dark due to the formation of autooxidation products of caffeic acid deriving from addition and/or polimerization side reactions [20].

Fig. 7 shows the concentration profiles of total caffeic acid obtained at various pHs under UV irradiation in the presence of O_2. The initial rate of photocatalytic degradation increased with increasing pH [17,26] indicating that the concentration of the dissociated species was the controlling factor of the reaction. The addition of TiO_2 enhanced the degradation of caffeic acid with respect to that observed in the absence of catalyst [26,32]. In particular, at each pH, the largest percentage of photodegradation was obtained in the presence of HP0.5 whereas the lowest value with Merck TiO_2. The different performances of the three samples were probably due to the differences among their specific surface areas and/or to the contemporaneous presence of rutile and anatase in P25 and HP0.5 [48].

Figs. 8 and 9 show the concentration profiles of *trans*-caffeic acid, *cis*-caffeic acid and esculetin, obtained at pH 6.0 and 8.0, respectively. At pH 3.5 the formation of *cis*-caffeic acid and esculetin was negligible and the concentration profiles of *trans*-caffeic acid were practically coincident with those of total caffeic acid reported in Fig. 7a.

Esculetin derives from the intramolecular cyclization of *cis*-caffeic acid. At pH 6.0 esculetin was obtained almost exclusively in homogeneous solution while at pH 8.0 the production of esculetin was observed both in the absence and in the presence of catalyst. At pH 6.0, in the presence of P25 and HP0.5, *trans*-caffeic acid was photodegraded without appreciable formation of *cis*-caffeic acid. Differently, in the presence of Merck TiO_2 or in homogeneous solution, the photodegradation process occurred along with the photoisomerization to *cis*-caffeic acid that was progressively consumed with formation of esculetin. At pH 8.0, *cis*-caffeic acid and esculetin were always detected in significant amounts during the runs performed with or without catalysts.

According to Le Person et al. [25], the photodegradation of *trans*-caffeic acid in homogeneous solution was the result of (i) the reversible *trans*/*cis* isomerization, (ii) the formation of esculetin from the *cis*-isomer and (iii) other routes leading to the formation of oxidation products. At pH 6.0, the rates of the last processes were probably much higher in the presence of TiO_2 so that the formation of esculetin was negligible. Instead, at pH 8.0 the rate of photoisomerization was comparable to that of photooxidation of *trans*-caffeic acid and both *cis*-caffeic acid and esculetin were detected in the presence and in the absence of TiO_2.

The formation of esculetin is reported in literature as a photochemical process. Butler and Siegelman [27] found conversion of caffeic acid to esculetin by UV light irradiation of *trans*-caffeic acid in the presence of Fe^{3+} ions. van Sumere et al. [49] observed that Mn^{2+} ions and oxygen enhanced the conversion of caffeic acid to esculetin. A suggested mechanism involves the formation of *cis*-caffeic acid by UV irradiation of *trans*-caffeic acid followed by an oxidative reaction to a hydroxylated intermediate and final cyclization to esculetin.

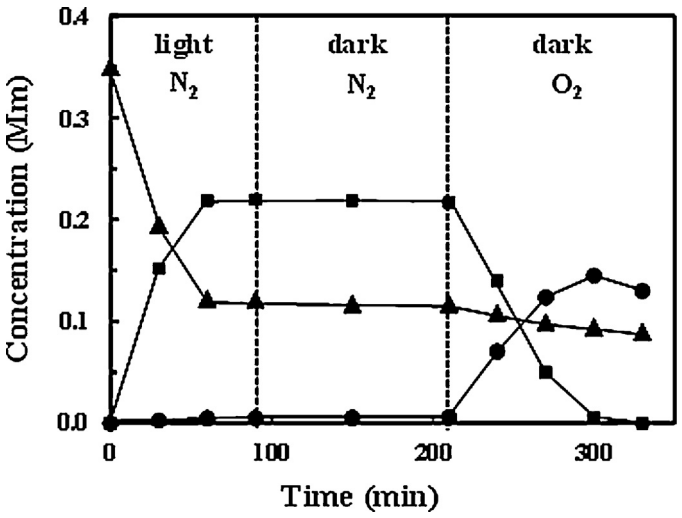

Fig. 10. Concentration profiles of *trans*-caffeic acid (▲), *cis*-caffeic acid (■) and esculetin (●) obtained with a 0.35 M *trans*-caffeic acid solution at pH 8.0.

This mechanism is generally accepted for the photodegradation of *trans*-caffeic acid [18,25,50]. Really, the formation of esculetin is not a "photochemical process" since it depends only on the availability of *cis*-caffeic acid. In fact, dark oxidation of solutions of pure *trans*-caffeic acid does not give rise to esculetin but to dimer and higher oligomers of caffeic acid [20] since the *trans*-isomer is geometrically unable to form a lactone. Otherwise, Painter and Neukom [51] obtained esculetin by dark oxidation of a solution of pure *cis*-caffeic acid with H_2O_2 and peroxidase. Dark oxidation of *ortho*-hydroxyquinones in the presence of molecular oxygen was also reported by Roginsky and Alegria [52].

Complementary experiments were carried out to confirm that the internal cyclization of *cis*-caffeic acid was not a light induced process. As shown in Fig. 10 photoisomerization runs were performed under nitrogen in the absence of catalyst until a photostationary state was reached. At this stage only traces of esculetin were detected in the reacting mixtures. Thereafter, the solutions were kept in the dark under nitrogen for 2 h in order to quench radicals possibly formed during the irradiation step. Successively, oxygen was bubbled in the solution and the concentrations of *cis*-caffeic acid, *trans*-caffeic acid and esculetin were monitored. The concentration of *cis*-caffeic acid continuously decreased and it completely disappeared within 2 h while the concentration of esculetin rapidly increased, reached a maximum value and then slowly decreased due to further oxidation. Contemporaneously, the concentration of *trans*-caffeic acid slowly decreased due to oxidative side processes. Similar results were obtained at pH 6.0 and 8.0.

Another control experiment allowed to confirm that the oxidation of *cis*-caffeic acid to esculetin did not practically occur at low pHs. A photoisomerization run was carried out at pH 6.0 under nitrogen for 2 h to obtain a mixture of *trans*- and *cis*-caffeic acid at the stationary state.The pH was adjusted at 3.5 and the solution was kept for 2 h under nitrogen in the absence of UV light. Finally, oxygen was continuously bubbled into the solution in the dark.

As shown in Fig. 11, the concentrations of *trans*- and *cis*-caffeic acid remained practically constant after 2 h and no esculetin was formed. These results allow to conclude that the oxidative cyclization of *cis*-caffeic acid is not only a photochemical process since it occurs also in the dark. The process is dependent on pH and the

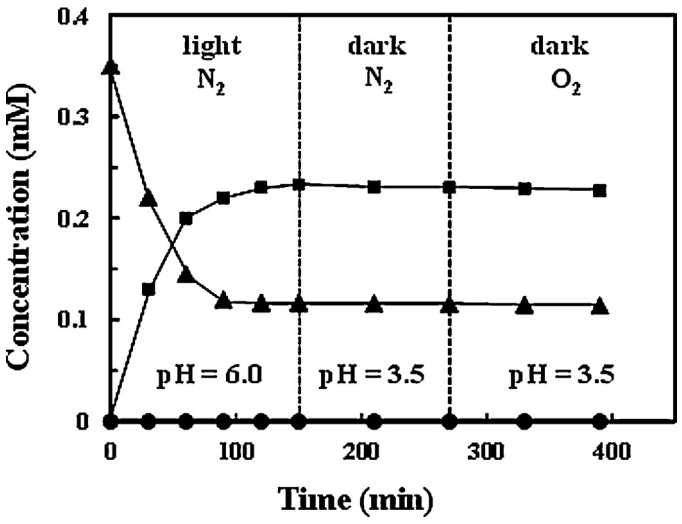

Fig. 11. Concentration profiles of *trans*-caffeic acid (▲), *cis*-caffeic acid (■) and esculetin (●) obtained with a 0.35 M *trans*-caffeic acid solution at pH 6.0. After UV irradiation under N_2, the pH was adjusted to 3.5.

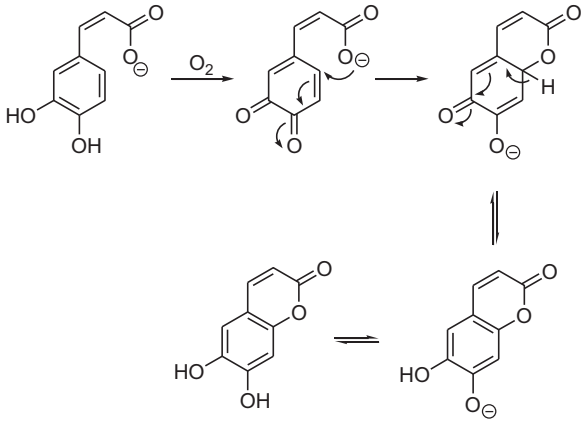

Fig. 12. Hypothesized mechanism for the formation of esculetin.

controlling rate factor is the caffeate ion concentration. A possible mechanism for the formation of esculetin is hypothesized in Fig. 12.

This mechanism justifies the absence of daphnetin (7,8-dihydroxycoumarin) since this last product cannot rise from a 1,4-nucleophilic attack. Moreover, at acidic pH the formation of esculetin is not favored because the protonated carboxylic acid is a too weak nucleophile to produce a nucleophilic addition to α,β-unsaturated systems.

4. Conclusion

Under irradiation, *trans*-caffeic acid is isomerized to *cis*-caffeic acid both in homogeneous aqueous solutions and in suspensions of TiO_2 catalysts. In the absence of oxygen, the degradation of *trans*-caffeic acid is suppressed so that the photoisomerization of the molecule is the only possible process. This study showed that the photocatalytic isomerization of *trans*-caffeic acid occurred through two synergistic parallel pathways: (i) photoisomerization in homogeneous phase and (ii) TiO_2-mediated photocatalytic isomerization. The results corroborated the hypothesis of an energy transfer process from TiO_2 to the substrate caused by electron–hole pair recombination. Experiments carried out in the presence of 2-propanol as a hole scavenger allowed to exclude an electron transfer dependent isomerization whereas modification of the TiO_2 surface with silyl groups enhanced the energy transfer pathway. In the presence of oxygen and at alkaline pHs, the photoisomerization of *trans*-caffeic acid is followed by an intramolecular cyclization to esculetin. This latter process occurs both in the absence or in the presence of irradiation according to a possible proposed mechanism.

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